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Review, Discussion, and Summary: Toxicology

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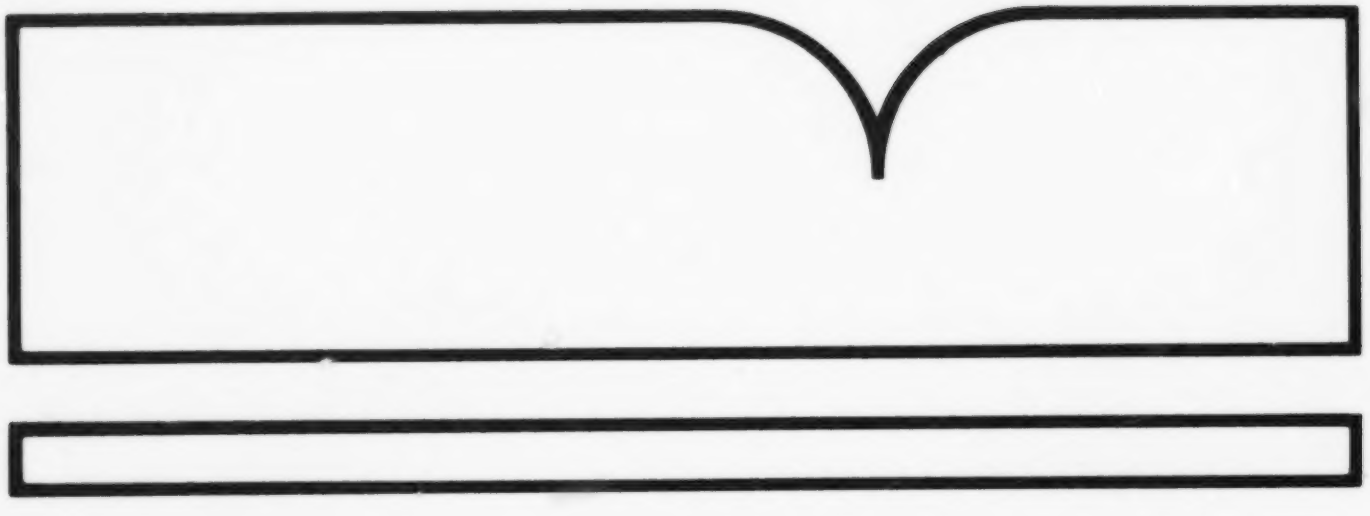

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16. ABSTRACT

The research presented in the toxicology session of the Symposium on the Health Effects of Acid Aerosols significantly advances our understanding of the health effects of acid aerosols and clearly illustrates the importance of animal inhalation toxicology to risk assessment. The description of the effects of acid on airway mucus buffering capacity and viscosity helps explain some of the mechanisms responsible for the effects of sulfuric acid on mucociliary clearance and pulmonary function observed in man and animals. Several of the papers illustrate that other pollutants interact with sulfuric acid (H_2SO_4), causing concern about exposure risks and helping in elucidating the effects observed in epidemiology studies that have not yet been duplicated in a laboratory. For example, H_2SO_4 absorbed on zinc oxide (ZnO) particles appears to be about a log more potent than H_2SO_4 alone in causing pulmonary function decrements. Low levels of H_2SO_4 and O_3 were found to be synergistic in increasing collagen synthesis, implying a risk in development of lung fibrosis. More complex mixtures containing H_2SO_4 cause a variety of interactions, depending upon the end points examined and the chemistry of the mixture. Other reports indicate that dose rate and length of exposure issues are critical to toxicological outcomes. Animal data on mucociliary clearance, which parallels that of human data, was extended to show that concentration of exposure was more important than time of exposure in eliciting a response, although time played a significant role. A recent chronic study showed that H_2SO_4 caused effects that also can occur in the development of chronic bronchitis. Although the causal relationship between H_2SO_4 and chronic bronchitis remains to be proven, this chronic animal study, interpreted in context with shorter term human and animal studies of H_2SO_4 and with findings in the effects of cigarette smoke, raises concern. It is noteworthy that even after the H_2SO_4 chronic exposure ceased, there were some progressive changes, implying irreversible effects.

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Review, Discussion, and Summary: Toxicology

by Judith A. Graham*

The research presented in the toxicology session of the Symposium on the Health Effects of Acid Aerosols significantly advances our understanding of the health effects of acid aerosols and clearly illustrates the importance of animal inhalation toxicology to risk assessment. The description of the effects of acid on airway mucus buffering capacity and viscosity helps explain some of the mechanisms responsible for the effects of sulfuric acid on mucociliary clearance and pulmonary function observed in man and animals. Several of the papers illustrate that other pollutants interact with sulfuric acid (H_2SO_4), causing concern about exposure risks and helping in elucidating the effects observed in epidemiology studies that have not yet been duplicated in a laboratory. For example, H_2SO_4 absorbed on zinc oxide (ZnO) particles appears to be about a log more potent than H_2SO_4 alone in causing pulmonary function decrements. Low levels of H_2SO_4 and O_3 were found to be synergistic in increasing collagen synthesis, implying a risk in development of lung fibrosis. More complex mixtures containing H_2SO_4 cause a variety of interactions, depending upon the end points examined and the chemistry of the mixture. Other reports indicate that dose rate and length of exposure issues are critical to toxicological outcomes. Animal data on mucociliary clearance, which parallels that of human data, was extended to show that concentration of exposure was more important than time of exposure in eliciting a response, although time played a significant role. A recent chronic study showed that H_2SO_4 caused effects that also can occur in the development of chronic bronchitis. Although the causal relationship between H_2SO_4 and chronic bronchitis remains to be proven, this chronic animal study, interpreted in context with shorter term human and animal studies of H_2SO_4 and with findings in the effects of cigarette smoke, raises concern. It is noteworthy that even after the H_2SO_4 chronic exposure ceased, there were some progressive changes, implying irreversible effects.

Introduction

The goals of this discussion are to integrate the new findings with the existing data and link the toxicological studies with several clinical and epidemiological reports in other sessions. Through such integration, our understanding of the health effects of acid aerosols increases. This also leads us to identification of major research needs that will be useful to quantitative risk assessment. This discussion does not contain complete summaries of each paper.

The new information presented at the symposium goes further than ever before in suggesting convergence of health effects data from epidemiological, human clinical, and toxicological studies around high ambient levels of sulfuric acid (H_2SO_4). Convergence is critical to risk assessment since it enables the combined strengths of the three major experimental approaches to far outweigh the inherent limitations of each.

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The research presented in Session III illustrates the role of animal inhalation toxicology in contributing to assessments of human health risk. Through animal models, mechanisms of effects can be elucidated, thereby enabling us to link and interpret apparently unrelated observations and identify risk factors, as aptly demonstrated by Holma (*1*). Controlled chronic studies are only possible with animals and are essential if we are to understand potential chronic effects of a chemical and hypothesize the causative chemical(s) in epidemiological studies. Gearhart's work is illustrative of this (*2*). When a phenomenon is described in animals and man, for example, the effects of H_2SO_4 on mucociliary clearance, animal toxicology enables the parameters of the effect to be explored in complex designs more amenable to animal models. Schlesinger's study of the critical parameter of the concentration (C) × time (T) relationship, as well as the relative influence of acidity (*3*), is indicative of this attribute of animal toxicology. In the environment, man is exposed to a complex mixture of pollutants that can only be studied fully with epidemiological approaches. However, since causative factors need to be understood in risk assess-

ment and management, complex mixtures need to be dissected and studied. Animal studies provide a valuable approach because they can use exposures to mixtures of unknown health risks and a wide array of end points that would create ethical problems for controlled human studies. Understanding gained by such research cannot only aid in interpretation of individual chemical and epidemiological studies, but can provide guidance to future research in animals and humans. The research papers presented by Amdur (*4*), Kleinman (*5*), and Last (*6*) fulfill this promise of animal toxicology.

The research presented in the Toxicology Session was exceptionally well designed to elucidate major uncertainties in understanding the health effects of acid aerosols. Therefore, the resulting data from all the studies can be integrated conceptually with each other, as well as with the existing data base. Thus, the organization of this discussion paper is purely arbitrary.

Basic Mechanisms of Effects

Holma's description of the effects of inhaled acids on airway mucus provides a fundamental basis for helping to understand the major mechanisms of acid effects (*1*). He showed that at low (and high) pH, mucus, and especially the glycoproteins in mucus, lose their buffering capacity, and the mucus increases in viscosity. Once the buffering capacity was saturated, the tissues would receive an increased dose of acid. The increase in viscosity would be expected to slow clearance, increase airway resistance (R_{aw}), and decrease diffusion capacity for carbon monoxide (DL_{CO}), all of which have been observed in humans and animals exposed to H_2SO_4 (*2–4,7–9*). In addition, the contribution of glycoproteins to mucus viscosity is partially dependent on ionic strength. Insofar as acid fogs are hypoosmotic (*10*), this mechanism may operate for a wide range of acid aerosols.

Holma's research raises another interesting and important issue: Are defense systems partially causal in toxicological responses? The buffering capacity of mucus clearly defends the underlying tissue by reducing the tissue dose of acids. Yet, as the buffering occurs, viscosity increases, which would presumably contribute to one of the major effects of acids, slowing of clearance. All crucial physiological systems have feedback control mechanisms to maintain homeostasis. It therefore could be speculated that the acid-induced decrement in mucus flow and saturation of buffering capacity might signal an increase in mucus production. This end event (increase in mucus production) is consistent with the increase in mucus-secreting cells observed morphometrically by Gearhart et al. (*2*). Other examples illustrate the delicate balance between defense and toxicity. A classical case occurs in the ozone (O_3) health effects data base (*11*). O_3 causes a decrease in phagocytosis of alveolar macrophages, inhibiting the capacity of pulmonary defenses against infections and nonviable particles. O_3 also stimulates an inflammatory response characterized by a relatively rapid (within 24–48 hr) influx of more alveolar macrophages and neutrophils into the lungs. Thus, there are more phagocytic cells to help maintain lung clearance capacity. However, these same cells are rich in proteases and under some experimental conditions can cause tissue destruction, including emphysema. In summary, inflammation has two competing roles—defense and toxicity. We do not yet possess sufficient understanding of the myriad of potential balances between defense and toxicity for acid aerosols or for other major pollutants, but there is sufficient information to pose hypotheses, the testing of which is important to understanding health risks.

Interactions of Acid Aerosols with Other Pollutants

Man is exposed to complex mixtures. Epidemiological approaches are the best way to study this exposure, but epidemiological studies are inherently confounded, and cause-effect relationships are difficult or impossible to establish. Controlled human and animal studies overcome this problem, but present another problem in that they are oversimplified with respect to the real-world complexity. Several researchers in this symposium attempted to strike the balance through the use of animal inhalation toxicology studies.

Amdur and her colleagues (*4*) exposed guinea pigs to the combustion reaction products of zinc oxide (ZnO) and sulfur dioxide (SO_2). The products were ZnO with a surface layer of H_2SO_4 and a residual gas phase of SO_2. ZnO and SO_2 alone at equivalent (and even somewhat higher) exposure levels were without effect. However, the mixture caused a number of pulmonary function effects, edema, and inflammation. Their most provocative finding was that surface-coated H_2SO_4 was approximately a log more potent than an equivalent H_2SO_4 mist for effects on DL_{CO}. The lowest effective exposure of surface coated H_2SO_4 was 20 μg H_2SO_4/m^3 (3 hr/day for 4 days of exposure). The mechanism for the enhanced potency is not known; perhaps the presence of SO_2, the relationship between the number of particles versus the mass of particles, the nature of the surface on which the H_2SO_4 was adsorbed, and/or the surface areas of the particles played a role(s). If this potentiation extends to other end points and to man, the implications are a matter of concern. For example, some human clinical studies show effects on pulmonary function and tracheobronchial clearance around 100 to 200 $\mu g/m^3$ H_2SO_4 (*7–9*). In the environment, it can be expected that some acids will be adsorbed on particles. If the effective concentration in these human studies was reduced by a log to 10 to 20 $\mu g/m^3$, this is within the range of more commonly encountered exposure conditions. These linkages are speculative at this time, given the paucity of exposure

synergistic, additive, or antagonistic (6,9). The synergism (6) with O_3 is of more concern because it has implications for the development of lung fibrosis. The interaction with NO_2 has been studied more rarely (3) and is too complex at this time to permit speculation.

The second hypothesis is that H_2SO_4 might cause chronic bronchitis (14). The hallmarks for chronic bronchitis are excessive mucus production and airway narrowing. This is associated with slowing of lung clearance. Such effects have been observed after chronic exposure of rabbits (2), donkeys (15), and monkeys (12). The linkage to man is derived from mucociliary clearance studies in rabbits (16), donkeys (15), and man (7,17,18) exposed to H_2SO_4 and in donkeys and man exposed to cigarette smoke (19). Briefly, donkeys exposed to cigarette smoke or H_2SO_4 respond similarly, with erratic changes in mucociliary clearance after repeated exposures; some donkeys had persistently slowed clearance. Donkeys, rabbits, and man exposed acutely to H_2SO_4 and cigarette smoke have similar responses, namely an acceleration of mucociliary clearance at low concentrations and a slowing of clearance at higher concentrations. Prolonged cigarette smoking causes chronic bronchitis in man. All these linkages between several animal species and man for several hallmarks of chronic bronchitis do not prove that H_2SO_4 causes chronic bronchitis. Nevertheless, they do support a strong hypothesis for the causation of bronchitis which needs to be tested experimentally.

This article has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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assessment and health assessment data. However, there are potential implications.

Last presented recent extensions of his interaction studies with aerosols and oxidants on collagen metabolism (*6*). He found that H_2SO_4 and O_3 were synergistic in increasing collagen synthesis in rats at exposures as low as 20 μg H_2SO_4 and 0.2 ppm O_3 (9 days of exposure). Previously, Last and others have shown that the methods applied here have implications to the development of lung fibrosis (*11*).

Kleinman presented the most complex controlled exposure study (*5*). The researchers exposed rats to the complex dark reaction products of various combinations of O_3, SO_2, nitrogen dioxide, H_2SO_4, ammonium sulfate, and catalytic metals. The results are too extensive to summarize here. One of the more novel aspects of the study was an examination of the upper respiratory tract, which is rarely included in protocols. Kleinman found that mixtures including H_2SO_4 increased cell death and cell turnover in the upper respiratory tract. This might increase our understanding of the health effects of acid fogs, which have larger particle sizes than more typical ambient acid aerosols. Sometimes when risks are assessed, the focus is on the lower respiratory tract and therefore larger size particles are somewhat discounted in terms of risk. Kleinman's research indicates that the upper respiratory tract is a target site of toxicity. Unfortunately, we know so little about health effects of this region that interpretation of degree of risk cannot be more than qualitative at this time.

Time of Exposure

Dose of a chemical at the target site is the predominant factor in toxicity. However, dose is one of the most poorly understood components of toxicity. The difficulty is due to the complexity of dose-rate issues. A full discussion of this issue is beyond the scope of this paper. Several investigators who reported on their research at this symposium recognized the critical nature of dose-rate and length of exposure if the results of controlled studies are ever to be quantitatively understood in the context of real-world exposures.

Schlesinger and his colleagues (*3*) studied mucociliary and alveolar clearance in rabbits exposed to H_2SO_4. After acute or short-term (days) exposures, at lower concentrations, clearance was accelerated; at higher concentrations, it was slowed. Similar relationships have been observed in humans (*7–9*). This body of work clearly illustrates the importance of using a range of concentrations in controlled studies and the immense difficulty (if not impossibility) of extrapolating from very high concentrations often found in the animal toxicological literature to low concentrations within the ambient range. Schlesinger also performed concentration × time ($C \times T$) studies using mucociliary clearance as an indicator. He found that although concentration of H_2SO_4 was more controlling of the response, time of exposure made a very substantial contribution. Through such results, we also understand the importance of monitoring studies that have short time-weighted averages if quantitative risk assessment is to be accomplished.

Gearhart reported on a study in which rabbits were exposed to 250 μg/m^3 H_2SO_4, 1 hr/day, 5 days/wk, for 12 months and examined periodically during exposure and 3 months postexposure (*2*). Some of the more interesting findings included an increased number of mucus secretory cells that shifted to smaller airways, a decrease in pH of the contents of the secretory cells, and a shift within the lungs to a larger proportion of smaller airways. These findings are consistent with the earlier chronic studies of Alarie et al. (*12*). In monkeys exposed to about 0.9 mg/m^3 H_2SO_4 plus 1 ppm SO_2 concentrations, there was goblet cell hyperplasia, bronchiolar epithelial hyperplasia, and thickening of the walls of respiratory bronchioles. Gearhart et al. also discovered that tracheobronchial clearance slowed with prolonged exposure, and slowed further even after H_2SO_4 exposure ceased, implying progressive, irreversible effects. Progressive effects have also been observed in dogs exposed to a mixture of H_2SO_4 and SO_2 for 5 to 6 years and examined 2 years postexposure (*13*).

Amdur's research also has a bearing on the length of exposure issue (*4*). Guinea pigs exposed to H_2SO_4 adsorbed on ZnO particles (3 hr/day up to 5 days) were examined for several end points, including total lung capacity, vital capacity, DL_{CO}, protein in lavage fluid, and number of neutrophils in lavage fluid. The pattern of the response relative to the number of days of exposure was dependent upon the end point and the concentration of H_2SO_4. For example, at 20 μg/m^3 H_2SO_4, DL_{CO} was decreased after 4 days of exposure; at 30 μg/m^3 H_2SO_4, the decrease was observed on day 2. The number of neutrophils peaked on day 3 of exposure. This research clearly illustrates that evaluating toxicity through a narrow window (i.e., one concentration, one time of exposure, one endpoint, one observation time) will not accurately reflect the health effects likely to occur in the real world.

Summary

The studies presented in this session, when taken in context with the existing literature, yield two major hypotheses about the potential risks of H_2SO_4. It must be emphasized that these are hypotheses that must be tested before they can be used in quantitative human health risk assessment. Whether similar hypotheses exist for other acid aerosols is speculative now and must await the development of more knowledge about the basic mechanisms of action of H_2SO_4 and effects of other acidic species.

The first hypothesis is that H_2SO_4 can interact with other common pollutants such as O_3 and nitrogen dioxide (NO_2). With O_3, these interactions have been